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Root allocation and water uptake patterns in riparian tree saplings: Responses to irrigation and defoliation

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Abstract

The genus *Populus* relies on shallow groundwater for successful recruitment and is often the focus of riparian restoration efforts. Under some circumstances mature trees take up a substantial proportion of their water from unsaturated soil water derived from growing season rainfall, but it is unknown how *Populus* saplings may alter root allocation patterns and water use in response to water availability and carbon limitations. Although it has been inferred that root allocation differs with changes in water uptake patterns as determined with stable isotope studies, this notion has rarely been tested. We conducted a glasshouse experiment with *Populus fremontii* (Frémont cottonwood) saplings to determine how allocation to fine and coarse roots, leaf gas exchange, root respiration and water uptake from hydrologically isolated upper and lower soil compartments would be altered by above- and belowground resource limitations. Aboveground carbon limitations were imposed with defoliation. Belowground resource limitations were imposed by maintaining high or low soil water availability in lower soil compartment. Isotopically labeled water was supplied in pulses to upper soil compartments to determine the proportion of transpiration water derived from each compartment. Above- and belowground resource limitations differentially altered use of surface water pulses and affected patterns of fine root allocation. Proportional use of water sources was plastic and changed in response to water availability and defoliation. Changes in fine root biomass allocation were associated with changes in water-source use for water-stressed plants. Defoliated plants in both watering treatments used proportionally less of the surface pulse than undefoliated plants. In contrast, plants that were water limited, but not carbon limited had a higher ratio of shallow fine roots to deep fine roots and took up proportionally more water from the surface pulse. These data suggest that carbon limited saplings take up less water with shallow roots. Thus, *P. fremontii* exhibited belowground allocation tradeoffs in response to spatial heterogeneity of soil water and carbon limitations. Furthermore, our data suggest that successful recruitment events may be influenced by the occurrence of summer rainfall, and by factors affecting canopy carbon gain.
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Keywords: *Populus fremontii*; Root respiration; Stable hydrogen isotope ratios; Water-source use; Rhizopods

1. Introduction

There has been considerable research focused on the genus *Populus* and its reliance on groundwater. Several studies have examined the effects of different rates of groundwater decline on the survival and growth of cottonwood using experimental pots ('rhizopods'), which simulate groundwater decline (Mahoney and Rood, 1991; Amlin and Rood, 2002). In general, shoot growth is reduced with increasing rates of water table decline, from 1 to 10 cm day^{-1}, and this results in an increased root:shoot ratio (Mahoney and Rood, 1991, 1992; Kranjcec et al., 1998; Amlin and Rood, 2002). Root elongation rates are generally greatest with moderate (2–4 cm day^{-1}) water table declines (Mahoney and Rood, 1992; Kranjcec et al., 1998; Amlin and Rood, 2002), however in some cases severe drawdown (10 cm day^{-1}) promoted the greatest rates of root elongation (Mahoney and Rood, 1992; Kranjcec et al., 1998; Amlin and Rood, 2002). The highest rates of seedling survival have been found for moderate drawdown rates (Mahoney and Rood, 1991; Segelquist et al., 1993), which varied from 2 to <1 cm day^{-1} for hybrid *Populus* in Canada and *Populus deltoides* in Colorado, respectively. Optimal rates of drawdown for root elongation and seedling survival are likely to vary with climate, species and soil substrate (Kranjcec et al., 1998).

Few studies have examined whether plants may be able to substitute shallow soil water for groundwater in these

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controlled experiments. In many riparian systems summer monsoon rains during the height of the growing season could provide alternative sources of water to promote successful recruitment events. The recruitment box model (Mahoney and Rood, 1998) integrates rates of stream hydrograph decline with cottonwood seed viability and root elongation rates to predict successful recruitment events, which is useful for restoration. For example, a 2.5 cm day^{-1} groundwater decline facilitates successful recruitment of cottonwood along the Truckee River in Nevada. Yet other sources of water may be available to both native and non-native species, which may impact recruitment events and the applicability of the recruitment-box model if species are able to substitute precipitation for groundwater. Understanding what abiotic and biotic conditions promote use of shallow and deep water sources by woody plants and characterizing responses by species and populations is critical for establishing predictive relationships under future climate change scenarios or changing groundwater levels.

The primary objective of this study was to determine how root allocation patterns and use of shallow and deep water varied with drought and defoliation of *Populus fremontii* (Frémont cottonwood). Above- and belowground limitations were imposed by reducing carbon assimilation through defoliation and by changing belowground water availability.

Many woody plants in semi-arid and arid ecosystems have a dimorphic root system with shallow lateral roots and deep sinker or taproots. It is generally thought that plants proliferate roots in resource rich patches to optimize carbon allocation (see review by Fitter, 1994). In regions where water is often the most limiting resource to plant growth, proliferation of roots into moist patches carries a cost to the plant. In addition root growth into one patch comes as a tradeoff to growth into another moist patch. As a result if the moist patch is short lived, the cost of growing and maintaining the new root may not be repaid (Nobel et al., 1992; Fitter, 1994). Furthermore, as a plant becomes increasingly carbon-limited, there may be additional tradeoffs in resource allocation.

Traditional studies of roots using belowground harvesting have equated root proliferation with resource use from a particular patch (Fitter, 1994). However, these studies did not measure water or nutrient use directly. Recent studies of the stable isotopic composition of xylem sap have been used to directly determine water-source use from different soil depths (Brunel et al., 1995). These studies indicate that woody plants may redirect limited carbon resources in response to the relative availability of water resources (Dawson and Ehleringer, 1998; Kolb et al., 1997; Snyder and Williams, 2000). Changes in the isotopic composition of xylem sap have been used to "imply" that there are changes in rooting distribution or functional root area profiles. However, few studies to date have investigated whether changes in root biomass or distribution are associated with changes in water-source use (but see Dawson and Pate, 1996).

The objective of the current study was to determine if changes in root biomass distributions were associated with different water-use patterns within a controlled glasshouse environment and what abiotic and biotic conditions may promote use of shallow soil water. Additionally, we were interested in establishing some baseline data on *in situ* respiration of suberized and unsuberized roots of *P. fremontii*. We used *P. fremontii*, a woody phreatophyte, as a model plant because field studies have shown that this species can use different amounts of shallow soil water at sites with different depths to groundwater (Snyder and Williams, 2000) and because of the widespread interest in restoring cottonwood forests in the western United States. We asked the following questions: (1) does the presence of a stable deep water-source affect a plant's ability to use pulses of shallow soil water; (2) do plants exhibit plastic responses to changes in water availability belowground and are these changes manifested in root biomass patterns and water-source use; (3) does defoliation affect patterns of biomass allocation and pulse use; (4) what are the respiration rates of suberized and unsuberized roots? We predicted that plants with access to a stable deep-water source would optimally allocate to roots in wetter soil layers, and that this allocation would come as a tradeoff to root allocation in drier soil layers. Plants without a stable deep-water source were predicted to exhibit increased use of shallow soil water. As plants became increasingly carbon-limited we predicted they should exhibit more efficient foraging for water due to potentially greater respiratory costs of highly absorptive unsuberized roots.

2. Methods

2.1. Experimental design and pot construction

We constructed pots (i.e. rhizopods) to create different water availability in upper and lower compartments. The objective was to create similar soil moisture conditions in the upper compartments and different conditions in the lower compartment. Pots were constructed from polyvinyl chloride tubes 0.48 m in diameter and 1.1 m deep (Fig. 1). Pots were split in half to separate upper and lower portions of the soil column and

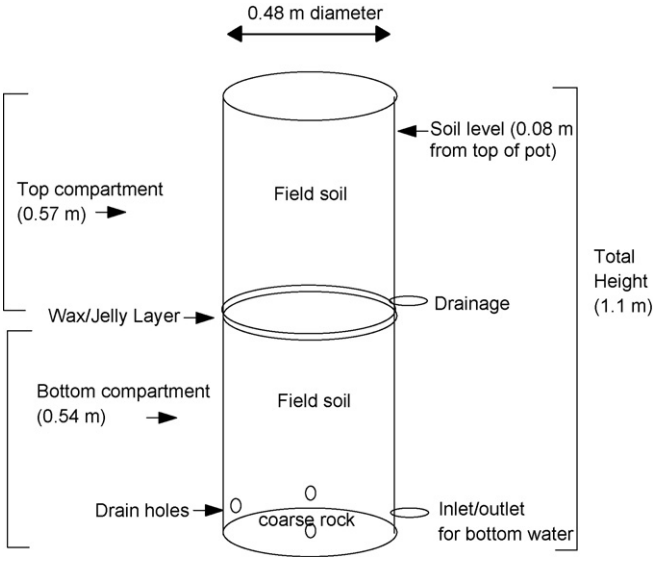

Fig. 1. A schematic of the pot design used in the glasshouse experiment.

bolted back together to facilitate removal of root biomass at the end of the experiment; seams were sealed with silicone to prevent leakage. A 0.05-m layer of coarse gravel was placed at the bottom and this was lined with a mesh screen to permit drainage and aeration at the bottom while minimizing soil loss through the drain holes. A clayey-sand field soil obtained from an old river channel in south Tucson (Triple A Fertilizer, Tucson, AZ) was mixed with time-release nutrient pellets (Sierra 17-6-10 with micronutrients, Scotts Chemical Co., Marysville, OH) to maintain adequate levels of nutrients. Pots were filled with this soil to a height of 0.53 m and then flooded from the bottom and allowed to drain and settle to remove macropores. After drainage, more soil was added to ensure pots were filled to a specified height of 0.53 m. A melted paraffin wax and petroleum jelly layer (1:6.5 by mass, wax:jelly) was poured into each pot and allowed to cool (Bryla et al., 1997). This layer was approximately 1.5 cm thick and prohibited water exchange between the top and bottom of the pot, but allowed unimpeded root growth. The remainder of the pot (0.49 m) was filled with the same field soil to within 0.08 m from the top. A drain-hole through the polyvinyl chloride tube in the upper compartment allowed drainage from above the wax layer. The bottom compartment contained three large drain-holes at the pot bottom that were sealed during bottom irrigation with rubber stoppers and one other inlet/outlet hole that connected via tubing to the central reservoir. Thus, pots could be watered from the bottom, through a large central reservoir (Mahoney and Rood, 1991). Four small holes drilled through the pot below the wax layer facilitated gas exchange in the lower compartment. Pots were planted with cuttings of *P. fremontii*, obtained from a nearby field site (see Snyder and Williams, 2000).

Plants initially grew under similar conditions and received ample water from the top and bottom of the pots. Greenhouse temperatures were between a minimum of 20° and a maximum of 30 °C. When plants no longer wilted with the removal of surface water, roots were assumed to have penetrated to the lower compartment and treatments were initiated. Watering and defoliation treatments, as explained below, were initiated 150 days after planting and continued until the final harvest.

All combinations of two treatments, watering and defoliation, were employed. All plants were given 1.25 l of water every other day from the top of the pots throughout the experiment, administered via a drip irrigation system. This water was supplied to plants to maintain live roots in the tops of pots. The amount of water supplied to the lower compartment differed. Watering treatments consisted of a dry lower compartment or a wet lower compartment. The wet treatment maintained a high water content in the lower compartment with weekly flooding from the central reservoir. The dry treatment was wet thoroughly once, and then allowed to dry-down until just prior to each sampling date.

Leaf removal treatments consisted of undefoliated and defoliated plants. Defoliated plants had half of their leaves removed by hand every 2 weeks. The experimental design was a replicated fully factorial experiment, with 12 individuals randomly assigned to all combinations of bottom water conditions and leaf removal: (1) dry-undefoliated, (2) dry-defoliated, (3) wet-undefoliated and (4) wet-defoliated ($n = 3$ per treatment combination).

2.2. Measurements

After plants grew under treatment conditions, they were pulsed (irrigated) with water labeled with deuterium ($\delta D \approx 182‰$) at the soil surface to determine which plants used the pulse of shallow soil water. *P. fremontii* plants were sampled for isotopic composition of xylem sap according the following protocol 13 and 19 weeks after initiation of treatments. Three days before sampling, normal maintenance watering in the top compartment was ceased. Predawn leaf water potential (Ψ_{pd}) values were measured with a Scholander-type pressure chamber. Then the lower compartments of all plants were fully saturated and allowed to drain to minimize water limitations in the lower compartment (hereafter referred to as irrigation), 24 h before the application of the surface pulse. We were interested in assessing the effects of water availability and defoliation on belowground biomass allocation, not the effect of drought on transpiration, therefore when assessing response to surface pulses all plants were provided ample water in the bottom compartment.

Plant stems of approximately 0.5 cm diameter were sampled prior to surface irrigation from sunlit branches and stored in airtight glass vials for subsequent analysis of hydrogen isotope ratios (δD). Deuterium-enriched water was mixed in a large container and then hand-applied to the soil surface. To minimize ponding at the soil surface and preferential flow, the 3-l surface pulse delivered to each plant was applied in the form of 1 l every 2 h beginning at 9:00 h. The next day, between 9:00 and 10:00 h, plant stems were sampled for isotopic analyses, as described above. In the laboratory, water was extracted from plant stems by cryogenic vacuum distillation (Ehleringer and Osmond, 1989; Smith et al., 1991). Water extracted from plant stems and irrigation water were analyzed for hydrogen isotope ratios (δD) using a dual inlet isotope ratio mass spectrometer with a precision of ±0.9‰ (Delta-S, Finnigan-MA, Bremen, Germany). A chromium reduction furnace attached on-line to the mass spectrometer was used to convert liquid water to hydrogen gas (HDevice, Finnigan-MAT, Bremen, Germany). δD values are reported relative to V-SMOW. Laboratory standard waters were used for correction to V-SMOW.

Transpiration was measured continuously during isotopic labeling with heat balance collars (Dynamax Inc., Houston, TX). Gas exchange was measured with a portable infrared gas analyzer (PP Systems, CIRAS-1, Herts, UK) immediately prior to irrigation of the lower compartment and the surface pulsing, and after all plants were well-watered. For gas exchange measurements CO_2 was maintained +5 ppm above ambient and relative humidity and light conditions followed ambient conditions.

In situ root respiration was measured before plants were harvested. Pots were pushed over and unbolted to expose roots (Fig. 2). Roots were carefully cleaned of soil. Then sterilized by spraying intact roots with a NaOCl solution (0.5% by weight)

Fig. 2. Exposed roots of *Populus fremontii* at the end of the experiment. The wax–jelly layer impeding water exchange between the bottom and top compartment, but allowing root growth, is visible. Differences in soil moisture are apparent.

waiting 1 min and rinsing with distilled water (see Nobel et al., 1992). Intact roots were patted dry and then placed inside a cuvette and respiration was measured with a CIRAS infrared gas analyzer (PP Systems, Haverhill, MA). The cuvette was covered with foil to measure dark respiration and isothermal conditions in the cuvette head were maintained by attaching a 25 °C water bath and flowing it through the outer housing of the cuvette. Relative humidity tracked ambient humidity, flow rate was 150 μmol s^{-1}, CO_2 was maintained at 465 ppm (to account for higher levels of CO_2 within the glasshouse) and roots were allowed to equilibrate for 10 min at which time the measurement was taken. This was done for two suberized and two unsuberized roots on each individual. Measurements were averaged so there was one measurement per plant for suberized and unsuberized root. Roots within the cuvette were then excised, dried and weighed. Respiration is expressed on a weight basis as nmols of CO_2 per gram of root per second.

After the final pulsing experiment, pots were opened along the length of the pot and roots were washed and separated into fine (<5 mm in diameter) and coarse (>5 mm in diameter) fractions within upper and lower compartments. All above- and belowground biomass was harvested, oven-dried and weighed.

2.3. Data analyses

Two-way analysis of variance (ANOVA) was used to assess the effects of watering and defoliation on three response variables. The three response variables were photosynthetic rate (A), Ψ_{pd} values immediately before irrigation, and the difference between xylem δD values immediately before surface watering and after irrigation with labeled water. If a significant interactive effect was present, contrasts were used to determine which treatment combinations differed. The change in δD values was used because it reflects the result of the surface pulse, which minimizes differences in the initial δD values that were a function of differences within pots prior to the labeled surface pulse. Data were transformed as necessary to meet the assumptions of ANOVA based on inspection of residual plots. Non-transformed values are presented in the figures and text. Although plants were sampled at two different time periods, we choose not to use repeated measures because of the small samples sizes and lack of multiple time periods. We have limited our inferences to differences between treatments within a time period.

Mass ratios between various plant structures (shoots, fine roots, coarse roots) and ratios between roots in the two compartments as a proportion of total root biomass were examined to determine plasticity in allocation (McConnaughay and Coleman, 1999). Such ratios were used to standardize differences associated with ontogeny related to plant size. Ratios were constructed within each compartment as well as by summing upper and lower compartments. ANOVA models, as described above, were used to assess the effects of watering and defoliation on these ratios.

A two-compartment linear mixing model was used to calculate the proportion of xylem water derived from the surface pulse and deeper soil layers. The model was of the form:

$$f = \frac{\delta D_{p0} - \delta D_{p1}}{\delta D_{pulse} - \delta D_{tap}} \quad (1)$$

where f is the fraction of water derived from the labeled pulse water, δD_{p0} is the δD value of stem xylem sap prior to application of labeled water, δD_{p1} is the δD values of stem xylem water 1 day after application of labeled water, δD_{pulse} is the value of labeled water, and δD_{tap} is the δD values of the greenhouse tap water used to water plants, with the exception of labeled surface pulses. The proportion of water derived from deeper soil layers (Y) was calculated as

$$Y = 1 - f \quad (2)$$

Occasionally, negative mixing values were obtained (i.e. plants appeared to be using a more negative source of water than was present); in this case mixing values were assumed to be zero.

Transpiration on a per-unit sapwood area basis was partitioned by multiplying the proportion of water derived from shallow and deep soil with the average flow rate at midday (1000–1500 h). This quantified the absolute volume of water derived by plants from shallow and deeper soil layers.

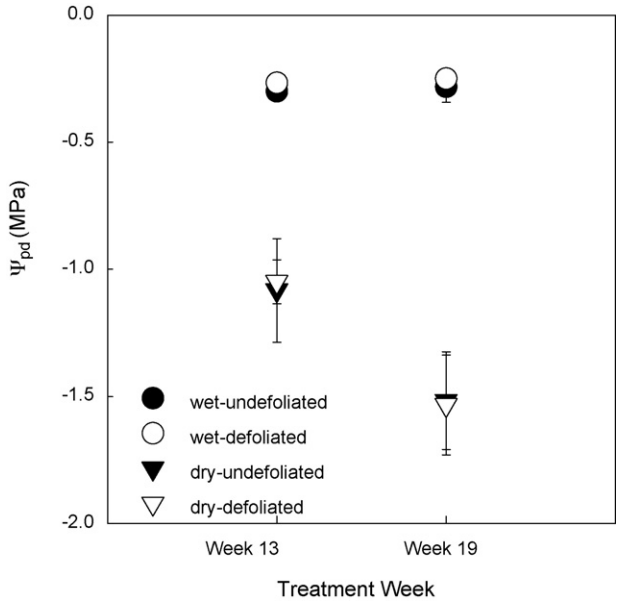

Fig. 3. Mean predawn leaf water potential (Ψ_{pd}) of *P. fremontii* for plants grown under different watering and defoliation treatments. Vertical bars represent ±1S.E. of the mean.

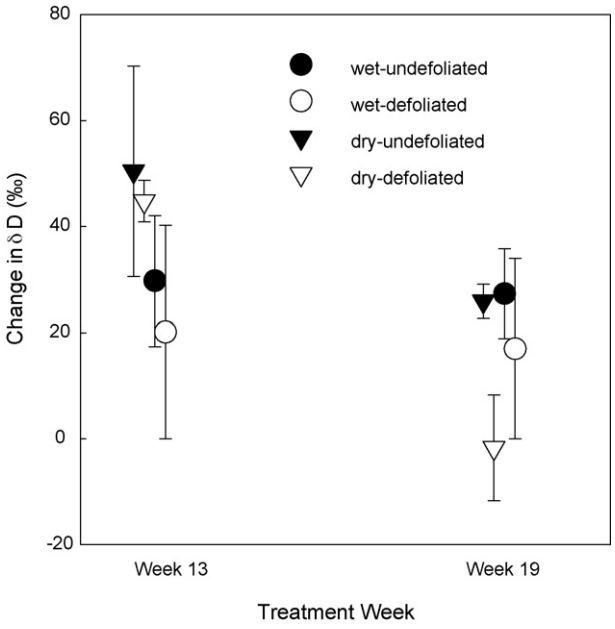

Fig. 4. The mean change (±1S.E.) in δD values of xylem sap of *P. fremontii* 1 day after surface irrigation with deuterium-labeled water. Plants were grown under different watering and defoliation treatments.

3. Results

3.1. Water potentials, gas exchange and water-source use

Predawn leaf water potentials (Ψ_{pd}) on both sampling dates measured before irrigation and surface pulsing were only affected by watering treatments (Fig. 3). As expected, Ψ_{pd} values were more negative in dry treatments than wet treatments, which indicates that there were indeed differences in available soil water between the two watering treatments. Photosynthetic assimilation rates (A) on all sampling dates were predominately affected by watering regime; with A being greater in wet treatments than dry treatments prior to irrigation and surface pulsing (Table 1). During treatment week 13, A increased after irrigation and surface pulsing in all treatments, except in wet-undefoliated plants, likely because water availability and A were high prior to irrigation and surface pulsing. Assimilation rates were able to recover when provided ample water in the other three treatments, however defoliated plants had greater increases in A than undefoliated plants, perhaps indicating some level of photosynthetic compensation (Nowak and Caldwell, 1984) by the remaining leaves. By treatment week 19, dry-defoliated plants experienced little increase in A after watering, apparently because these plants were unable to recover from the multiple stresses of defoliation and drought. Plants in the dry watering treatments had significantly lower A than plants in the wet treatments even after being provided with ample water in the bottom of the rhizopods.

The change in xylem water δD values after surface pulsing in treatment week 13 was similar for plants in all treatments ($p > 0.20$) (Fig. 4). Apparently all plants used a similar proportion of surface pulse water regardless of defoliation or watering and there were no interactive effects (Fig. 4). By treatment week 19, δD of undefoliated plants increased more than that in defoliated plants in response to the surface pulse. This effect of defoliation was only a weakly significant effect of defoliation, likely due to small sample sizes ($p = 0.10$) (Fig. 4). There were no interactive effects of defoliation and bottom water treatments ($p = 0.62$). Biologically, it appears that defoliated plants had less active water absorption in the upper soil compartment, with this trend being driven mainly by the dry defoliated treatment. Negative mixing values were calculated in a few instances and these values were set to zero;

Table 1
Mean (±S.E.) photosynthetic assimilation rates (A; μmol m^{-2} s^{-1}) of *Populus fremontii* plants grown under different watering and defoliation treatments

Weeks of treatment	Treatments				Treatment effects (p-values)		
	Wet-undefoliated	Wet-defoliated	Dry-undefoliated	Dry-defoliated	Watering	Defoliation	Watering × defoliation
Week 13 prior to irrigation	12.6 (0.3)	14.9 (1.6)	6.1 (1.6)	1.1 (0.6)	0.0001	0.15	0.07
Week 13 prior to irrigation	8.7 (2.4)	19.8 (0.42)	17.0 (2.1)	23.2 (4.5)	0.24	0.08	0.80
Week 19 prior to irrigation	16.4 (3.5)	12.5 (0.7)	1.9 (1.2)	2.2 (0.3)	0.0003	0.37	0.31
Week 19 after irrigation	18.2 (1.05)	20.3 (2.3)	5.8 (4.5)	2.9 (1.2)	0.004	0.76	0.60

Treatment effects were determined with a 2-way ANOVA model.

Table 2
Mean percentage of total transpiration derived from the deuterium-labeled surface pulse for *P. fremontii* plants grown under different watering and defoliation treatments

Weeks of treatment	Treatments			
	Wet-undefoliated	Wet-defoliated	Dry-undefoliated	Dry-defoliated
Week 13	26.8 (11.1)	52.1 (18.1)	45.3 (17.9)	40.3 (3.5)
Week 19	24.0 (7.4)	13.3 (13.3)	22.7 (2.9)	2.4 (2.4)

Values in parentheses are ±1S.E.

this was the case for two of the defoliated dry plants and two of the wet defoliated plants in treatment week 19. For the wet-defoliated plants slightly more negative values were observed after pulsing with positively labeled water, however these values were very close to the δD values observed prior to pulsing and may lie within the range of technique and instrument error. For the dry defoliated plants the δD values obtained after pulsing with labeled water were approximately −40‰ more negative than δD values prior to pulsing. However, the lack of isotopic enrichment indicates that these plants were not using labeled pulse water and therefore it is reasonable to set the percentage of use of the surface pulse to zero.

Percent water-source use (Table 2), in conjunction with average maximum transpiration flux, gave an indication of the amount of water used from each compartment (Fig. 5). Wet-defoliated, dry-defoliated, and dry-undefoliated plants used approximately the same amount of deep water 13 weeks after treatment initiation, while wet defoliated plants used less deep water during peak transpiration (Fig. 5). The amount of shallow pulse use was approximately the same in wet-defoliated, wet-undefoliated and dry-undefoliated plants, while dry-defoliated plants used less pulse water. By week 19, plants in both wet treatments used a greater amount of deep water and shallow pulse water than plants in dry treatments (Fig. 5). Within the dry treatment, defoliated plants used less shallow water than undefoliated dry plants.

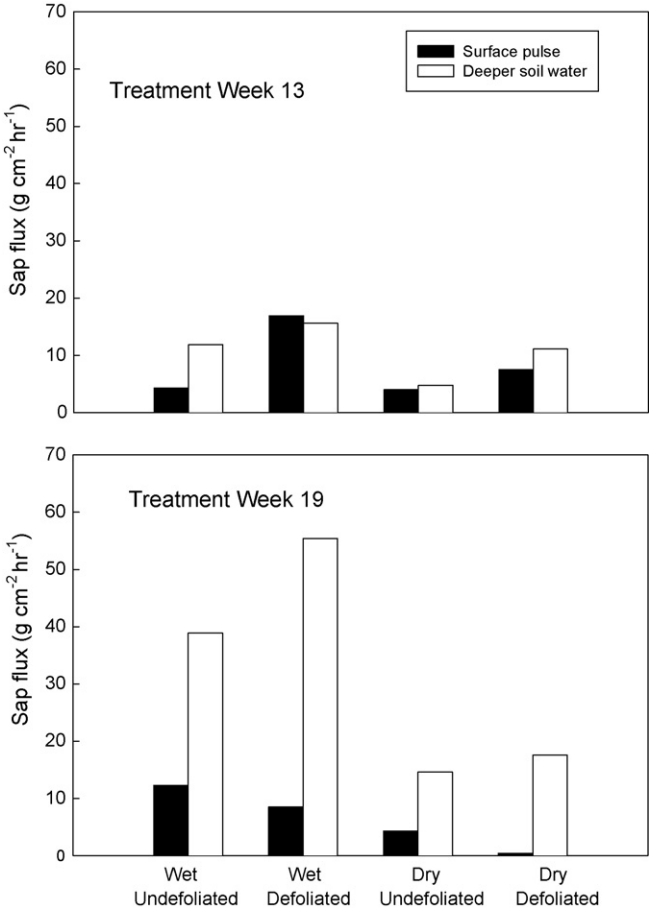

Fig. 5. Amount of transpiration of *P. fremontii* derived from the deuterium-labeled surface pulse and from water deeper in the soil. Data are expressed as transpiration flux (grams H_2O per unit sapwood area per unit time) at the scale of single plants. Values were derived by combining averaged rates of water flux at midday (1000–1500 h) with the percentage of water used from deep and shallow layers derived from isotopic data and the two-compartment-linear mixing model.

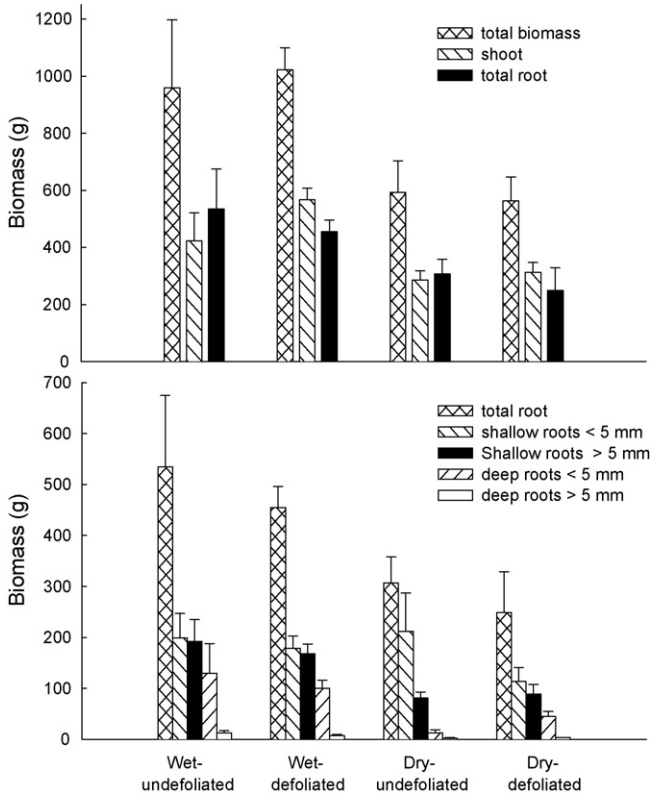

Fig. 6. Mean biomass (±1S.E.) of different plant structures for *P. fremontii* plants grown under different watering and defoliation treatments. "Shallow" indicates root biomass in the upper half of the pots and "deep" indicates root biomass in the lower half of the pots.

Table 3
Mean (±1S.E.) biomass ratios of different plant structures for *P. fremontii* grown under different watering and defoliation treatments

Variable	Treatments				Treatment effects		
	Wet-undefoliated	Wet-defoliated	Dry-undefoliated	Dry-defoliated	Watering	Defoliation	Defoliation × watering
Shoot:root	0.81 (0.07)	1.25 (0.07)	1.14 (0.21)	1.30 (0.12)	0.33	0.02**	0.58
All fine roots:total root biomass	0.60 (0.03)	0.61 (0.05)	0.70 (0.07)	0.63 (0.04)	0.21	0.58	0.81
All coarse roots:total root biomass	0.31 (0.03)	0.39 (0.05)	0.29 (0.07)	0.36 (0.04)	0.21	0.58	0.43
Shallow fine roots:total root biomass	0.38 (0.04)a	0.39 (0.04)a	0.67 (0.07)b	0.45(0.02)ab	N/A	N/A	0.04**
Shallow coarse roots:total root biomass	0.37 (0.03)	0.37 (0.04)	0.29 (0.06)	0.36 (0.04)	0.30	0.51	0.48
Deep fine roots:total root biomass	0.22 (0.05)a	0.22 (0.01)a	0.04 (0.02)b	0.19 (0.04)a	N/A	N/A	0.06*
Deep coarse roots:total root biomass	0.02 (0.007)	0.03 (0.007)	0.004 (0.004)	0.006 (0.006)	0.03**	0.52	0.33
Fine roots:coarse roots	1.53 (0.20)	1.65 (0.30)	1.83 (0.30)	1.83 (0.31)	0.22	0.45	0.34
Shallow fine roots:shallow coarse roots	1.03 (0.17)	1.10 (0.21)	1.76 (0.34)	1.29 (0.14)	0.11	0.22	0.18
Shallow fine roots:deep fine roots	1.96 (0.49)a	1.82 (0.01)a	13.07 (4.66)b	2.63 (0.54)a	N/A	N/A	0.01**

Treatment effects were determined with a two-way ANOVA model (*$p \leq 0.10$) (**$p \leq 0.05$). If a significant interactive effect was determined contrasts were used to determine which treatments differed and treatments differences are indicated by lowercase letters ($\alpha = 0.05$).

3.2. Biomass allocation patterns

Total biomass was reduced in dry treatments relative to wet treatments (Fig. 6) and data are presented for reference. However, to avoid the influence of differences in plant size, we used ratios to examine allocation patterns (Table 3). The ratio of total shoot to total root biomass increased in defoliated plants relative to undefoliated plants, indicating that defoliation produced a greater allocation to aboveground stems and leaves (Table 3). The total allocation to fine roots expressed as a proportion of total biomass and the total allocation to coarse roots expressed as a proportion of total biomass, did not change with drought or defoliation stress. Additionally, the ratio of fine roots to coarse roots in the top (shallow) compartment and aggregated across compartments (i.e. total fine roots:total coarse roots) was unaffected by watering and defoliation. In contrast to these patterns, allocation to fine roots was affected interactively by watering and defoliation. The ratio of shallow fine roots to deep fine roots increased for dry-undefoliated plants relative to other treatments ($p < 0.01$). Similarly, the ratio deep fine roots to total biomass decreased, and the ratio shallow fine roots to total biomass increased for dry defoliated plants. To determine if this increased allocation to shallow fine roots and reduced allocation to deep fine roots was simply a function of total plant size, the ratio of shallow fine roots to deep fine roots was plotted as a function of total biomass (data not shown). The slope of the regression was not significantly different from zero ($p = 0.54$). This indicates that this root allocation pattern was not due to differences in plant size across the various treatments.

Coarse root biomass in the bottom compartment was affected only by watering. Plants in wet treatments had a greater ratio of coarse root to total root biomass than those in dry treatments. Four of the six plants in dry treatments had no coarse roots in the lower compartment, while all plants in wet treatments had coarse roots in the lower compartment. In contrast, the ratio of shallow coarse roots to total root biomass in the upper compartment was unaffected by watering and defoliation.

3.3. Root respiration

There was no treatment effect of watering or defoliation on root respiration rates (data not shown). However a student *t*-test indicated that dark respiration rates of unsuberized white colored roots were significantly different than the respiration rates of suberized brown roots ($p < 0.005$). Unsuberized root respiration rates were nearly three times that of suberized roots. Dark respiration of unsuberized roots was 108 ± 13 nmol CO_2 g^{-1} s^{-1} (mean ± 1S.E.), whereas mean respiration in suberized roots was 42 ± 8 nmol CO_2 g^{-1} s^{-1}.

4. Discussion

There were three important findings from this experiment with *P. fremontii*. First, proportional water-source use of this species is flexible and changes in response to water availability and defoliation. Second, changes in fine root biomass allocation were associated with changes in water-source use for water-stressed plants. Third, defoliation reduced the proportion of shallow soil water used by cottonwood.

In terms of testing whether root biomass distribution was a good indicator of water-source use it appears that under well watered-low stress conditions root biomass distribution was not a good indication of water-source use. This species was able to maintain roots in multiple soil layers and use water opportunistically. Under stressful conditions, fine root distribution was more indicative of water-source use.

The respiration rates of suberized roots in this study were within the range of respiration rates of other temperate woody species (Eissenstat et al., 2000). *Populus tremuloides* roots had a respiration rate of approximately 55 nmol CO_2 g^{-1} s^{-1} (Reich et al., 1998), which agrees with our measured respiration rate of suberized roots in *P. fremontii*. Sustaining fine unsuberized roots for water and nutrient absorption apparently comes at a great cost, and thus allocation tradeoffs are to be expected when carbon is limited.

Plasticity in the allocation of carbon to root growth in *Populus* expressed itself on a relatively short time scale (i.e.

months) in this study. Other studies of the genus *Populus* found root production was responsive to environmental conditions such as atmospheric CO_2, soil N (Pregitzer et al., 2000; Zak et al., 2000), and water availability (Mahoney and Rood, 1991; Shafroth et al., 2000). In addition, we found that removal of leaves reduced root growth relative to shoot growth, and other studies also confirm a relationship between leaf and root growth for *Populus* (Pregitzer and Friend, 1996).

In the current study, lack of deep water reduced coarse root growth, and promoted redistribution of fine roots. Interestingly, the ratio of fine root to coarse root biomass, as well as the ratio of fine and coarse roots aggregated across upper and lower compartments, was unchanged. In contrast, there was a shifting of where plants grew fine roots when faced with stresses of defoliation and water limitations. Patterns of fine root growth, only appeared to explain differences in proportional water-source use in the water-stressed plants. This suggests that broad measures of root structure, such as total root volume and depth, may not adequately characterize plant water-source use, and that the ratio of fine roots to total roots in different soil layers may need to be considered.

Reduced availability of deep water produced differential responses depending on whether a plant was carbon-limited by defoliation. Dry-undefoliated plants, that likely had more carbon to allocate belowground than dry-defoliated plants, increased allocation to shallow roots when deep water was limited, but decreased allocation to deep fine roots. These changes in biomass were associated with changes in water-source use. These plants used a similar proportion of the surface pulse as plants with access to a reliable source of deep water, but at the expense of root development in deeper soil layers. This is consistent with other experimental work in which taproot desiccation promoted lateral root growth both in *Quercus* (Callaway, 1990) and *Salix* (Horton and Clark, 2001).

Dry-defoliated plants allocated more biomass to deep fine roots and had similar allocation to shallow fine roots as the plants in wet treatments, but less than in dry-undefoliated plants. Contrary to our original predictions, these plants did not use the surface pulse, which indicates that roots present in the shallow soil layers contributed little to plant water uptake by week 19. The increased allocation to deep roots by dry-defoliated plants may be a result of the way treatments were applied. The ''dry'' lower compartment, while drier than the ''wet'' treatment, was still likely wetter than the top compartment during the experiment. It seems plausible that dry-defoliated plants, which had the lowest A and likely the least photosynthate to expend, maintained fine roots in the lower compartment because it was still the wettest compartment. The shallow roots may have been produced earlier in the experiment and although apparently not substantially contributing to water uptake were still present in the soil at time of harvest. Although these roots appeared to be alive, it is possible that some were dead, or that some may not have been conductive to water due to presence of xylem embolisms (Sperry et al., 1998). Low water availability has been found to cause stem xylem cavitation in *P. fremontii fremontii* at fairly moderate water potentials (Tyree et al., 1994; Pockman et al., 1995).

For the dry defoliated plants the substantially more negative δD values which were set to zero are difficult to explain. In a separate study cottonwood trees at the height of the summer drought were found to have δD values more negative than the sampled environmental water sources (Snyder and Williams, 2000). It could be that there is an unknown mechanism in drought stressed trees that alters the isotopic composition of xylem water. For some halophytes and xerophytes the δD of plant xylem water can be depleted by 3–8‰ due to fractionation by plant roots (Ellsworth and Williams, 2007). However, it is unknown if this process occurs in the genus *Populus* and it this process could realistically explain the variation found in the current study. The lack of isotopic enrichment confirms that these plants were not using the highly labeled pulsed water and therefore it was reasonable to interpret that surface pulse water was not being used.

By the end of the experiment, all *Populus* plants except those in the dry-defoliated treatment used the shallow pulse of water. Our predictions, that dry-defoliated plants would use the highest proportion of the surface pulse, while wet treatments would use the lowest amount of the pulse were not supported. It appears the presence of a stable deep water-source allowed plants in wet treatments to maintain improved carbon assimilation between pulses of shallow water, and this carbon allowed plants to forage in multiple soil layers without facing allocation tradeoffs. Plants in dry treatments appeared to make allocation tradeoffs favoring increased allocation to deep roots, suggesting that there are tradeoffs in root biomass allocation.

The lack of surface pulse use in the dry-defoliated treatment is in direct contrast to two previous field studies on mature trees. In the first study cottonwood trees were found to use increasing amounts of shallow soil water with increasing depth to groundwater (Snyder and Williams, 2000). This highlights the need to understand carbon allocation behavior of different life history stages. In the second study, mature *Prosopis velutina* (mesquite) were defoliated and compared with undefoliated trees (Snyder and Williams, 2003). The defoliated mesquite trees became disconnected from the groundwater and more reliant on shallow soil water, whereas defoliated cottonwoods in the current study used porportionally more groundwater. This is perhaps another strategy that differs between facultative phreatophytes (e.g. mesquite) and obligate phreatophytes (e.g. cottonwood) in their ability to deal with water limitations.

Fine roots are important for plant water uptake because they are generally unsuberized and therefore highly permeable (Kramer and Boyer, 1995). It appears that growth of these fine roots may be very sensitive to changes in plant carbon assimilation. Perturbations to plant carbon assimilation, in conjunction with reduced water availability were manifested in shifting of fine root production to different soil layers in the current study. This shifting of fine root production resulted in differences in water-source use, which may reflect optimal foraging for water resources.

5. Management implications

The recruitment box model is based on the assumption that saplings rely on water stored in groundwater or the capillary fringe. The role of unsaturated (vadose) soil water is not specifically addressed. These results indicate that saplings in riparian environments may be able to use unsaturated soil water under some conditions. The most striking finding is that when saplings had ample water they were able to maintain roots in multiple soil layers. This means that in years with wet winters and wet summers the probability of successful recruitment events may increase. While this result seems obvious for a species classified as a phreatophyte, it suggests that more rapid rates of groundwater decline may be tolerated in years with high winter precipitation, if there is adequate summer precipitation. Though for purposes of restoration, predicting the occurrence of summer precipitation is notoriously difficult.

Additionally saplings that had a dry lower compartment (i.e. less available water), but were not defoliated allocated proportionally more to fine roots than in shallow layers, thus using more pulse water. This suggests that some rates of groundwater decline may promote use of shallow soil water; the caveat is that in the glasshouse we supplied constant and regular amounts of water to the top compartment of the rhizopod to maintain live roots. If summer rainfall is episodic and punctuated by long dry interpulse periods, saplings may not have the ''time'' to grow functional shallow roots and use rainfall. When extremely stressed (i.e. the dry defoliated treatment) this species was not able to use the shallow soil water.

If this species is able to use both groundwater and rainfall it may have a competitive edge over other species that rely solely on one water source. Whether other dominant riparian species or non-native species are capable of using multiple water sources at the sapling life history stage remains to be determined.

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